

Standard disc testing was used to determine antibiotic susceptibility and erythromycin resistance phenotypes. DNA from test samples was prepared by both Qiagen extraction and rapid boiling. As the efficiencies of the two methods were comparable, the rapid boiling method was used throughout. Primers were as published for *erm*(A), *erm*(B), *erm*(C), *mef*(A/E)⁵ and *erm*(TR).⁴ Donor and acceptor probe-pairs were designed for *erm*(A), *erm*(TR), *erm*(B), *erm*(C) and *mef*(A/E) respectively: ERMAADP1, CTGCAACGAGCTTTGGGTTTACTA and ERMAAP1, AATGGTGGAGATG-GATATAAAAATGC; DF-ERMA1, GTCAAGCGAAATATAG-CTACCTT and DR-ERMA1, TGTAGAGAGGGGATTGCTA; ERMA/MD1, CGTGTCACTTTAATTCACCAAGAT and ERMA/MA1, TCTACAGTTTCAATTCCTAACAAA; ERM/CD1, GTATGGTTCCAAGAGAATAT and ERM/CA1, TCATCCTA-AACCTAAAGTGAA; MEFA/ED1, TATCCGTAGCATTGG-AACAGCT and MEFA/EA1, TTCATACCCCAGCACTCAATG-CGGT. All donor probes were 3' end-labelled with fluorescein. Acceptor probes specific for *erm*(A), *erm*(B), *erm*(C) and *erm*(TR) were 5' end-labelled with LC Red 640; for *mef*(A/E), LC Red 705. Each acceptor probe had a 3'-phosphate blocking group.

Each 20 µL PCR mixture consisted of: 2 µL of FastStart DNA Master Hybridisation Probes (Roche Applied Sciences), 1.6 µL of 25 mM MgCl₂, 50 pmol of primers, 10 pmol of each probe pair, 8.4 µL of (PCR standard) water and 5 µL of bacterial DNA preparation. Two negative controls (PCR standard water and an erythromycin-sensitive GBS) were included in each run. Cycling conditions were: 95°C for 10 min, temperature transition rate (TTR) 20°C/s; 60 cycles 95°C for 0 s, TTR 20°C/s, annealing at 60°C for 10 s, TTR 20°C/s, and 72°C for 15 s, TTR 2°C/s; followed by a melting curve of: 99°C for 15s, TTR 20°C/s, 40°C for 15s, TTR 0.2°C/s to 95°C with continuous fluorescent acquisition. Fluorescence was measured on channel F2 for probes labelled with LC Red 640 and channel F3 for the LC Red 705-labelled probe.

Clinical isolates were tested four times using primers and probes specific for either *mef*(A/E), *erm*(A), *erm*(TR) or *erm*(B) genes. The *erm*(C) gene was not sought as its presence has only been reported in staphylococci.

Figure 1 shows the characteristic melting peaks for the positive controls; similar peaks were produced by the clinical isolates. *erm*(TR) and *erm*(B) were detected as the only erythromycin resistance genes in 41 (67%) and five (8%), respectively, of clinical strains with the MLS_B phenotype. All strains with the M-phenotype contained *mef*(A/E) only, while among those with the MLS_B phenotype, seven (11%) possessed double mechanisms of resistance: *erm*(TR) + *erm*(B), *erm*(TR) + *mef*(A/E) or *erm*(B) + *mef*(A/E). Two (3%) clinical strains expressing MLS_B phenotype were negative for erythromycin resistance genes. No isolate carried the *erm*(A) gene.

This assay, with the rapid boiling method for DNA extraction, provides results within 1 h of sample preparation. It is sensitive and specific, successfully identifying resistance genes in 97% of the erythromycin-resistant clinical strains of group B, C and G streptococci. With the increasing availability of Light-Cyclers, the assay can be used as a rapid tool for local, national and international surveillance of erythromycin resistance in streptococci, thereby guiding therapy.

In agreement with a previous study,⁶ we found *erm*(TR) to be the most frequent resistance gene. However, for definitive erythromycin resistance genotyping, isolates should be tested for the most common *mef* and *erm* genes, as more than one resistance

gene may be present.⁴ Many of the existing PCR assays are multiplex,^{3,4} reducing the number of reactions required. The characteristic melting peaks detected in the different channels are being exploited in developing a multiplex assay, which will reduce further the turn-around time and cost.

References

1. Leclercq R, Courvalin P. Resistance to macrolides and related antibiotics in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2002; **46**: 2727–34.
2. Health Protection Agency. *CDR Weekly*. Online. <http://www.hpa.org.uk/cdr/PDFfiles/2004/cdr1604.pdf> (March 2005, date last accessed).
3. Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L. Detection of erythromycin-resistant determinants by PCR. *Antimicrob Agents Chemother* 1996; **40**: 2562–6.
4. Farrell DJ, Morrissey I, Bakker S *et al.* Detection of macrolide resistance mechanisms in *Streptococcus pneumoniae* and *Streptococcus pyogenes* using multiplex rapid cycle PCR with micro-well-format probe hybridisation. *J Antimicrob Chemother* 2001; **48**: 541–4.
5. King A, Bathgate T, Phillips I. Erythromycin susceptibility of viridans streptococci from normal throat flora of patients treated with azithromycin or clarithromycin. *Clin Microbiol Infect* 2000; **8**: 85–92.
6. Kataja J, Seppala H, Skurnik M *et al.* Different erythromycin resistance mechanisms in group C and group G streptococci. *Antimicrob Agents Chemother* 1998; **42**: 1493–4.

Journal of Antimicrobial Chemotherapy

doi:10.1093/jac/dki316

Advance Access publication 2 September 2005

Loss of *mecA* gene in *Staphylococcus epidermidis* after prolonged therapy with vancomycin

Parham Sendi*, Peter Graber and Werner Zimmerli

Division of Infectious Diseases, Basel University Medical Clinic, Rheinstrasse 26, CH-4410 Liestal, Switzerland

Keywords: methicillin resistance, *S. epidermidis*, gene loss, glycopeptides

*Corresponding author. Tel: +41-61-925-34-19; Fax: +41-61-925-28-04; E-mail: sendi-pa@magnet.ch

Sir,

The most commonly cultured microorganisms in prosthetic-joint infections are coagulase-negative staphylococci.¹ Resistance to β-lactam antibiotics is encoded by the *mecA* gene; this gene is carried on a mobile genetic element, the staphylococcal chromosome cassette *mec* (SCC*mec*). Loss or deletion of the *mecA* gene rarely occurs, mainly due to factors affecting the stability of SCC*mec*. Vancomycin may induce deletion of the *mecA* gene in *Staphylococcus aureus*, as reported in this journal last year.² We report the case of an implant-associated infection due to a methicillin-resistant *Staphylococcus epidermidis* which lost the *mecA* gene after prolonged treatment with glycopeptides.

An 82-year-old man was admitted to our hospital because of a prosthetic-joint associated infection with a sinus tract, 4 months

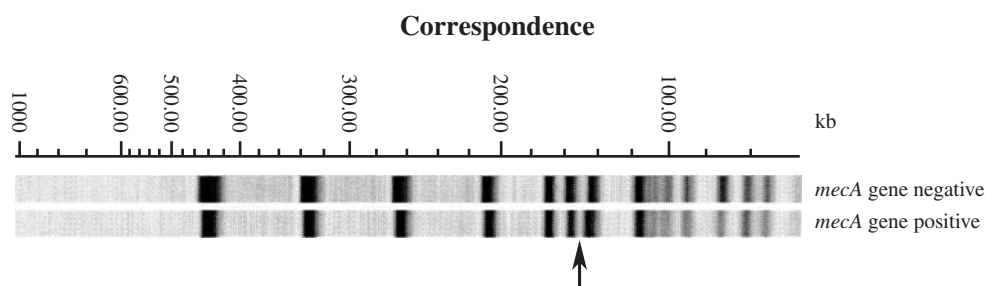


Figure 1. PFGE after *SmaI* digest: the distance between two bands at ~140 kb and at ~160 kb is shorter in the *mecA*-positive strain (arrow).

after implantation of a total hip prosthesis. *S. epidermidis* resistant to oxacillin, ciprofloxacin, trimethoprim/sulfamethoxazole, gentamicin, clindamycin, erythromycin, fusidic acid and rifampicin, but susceptible to tetracycline, teicoplanin and vancomycin grew in biopsies from periprosthetic tissue. Species identification was made using API ID 32 Staph system (bioMérieux, La Balme les Grottes, Montalieu Vercieu, France); the MIC of vancomycin was 2 mg/L determined by Etest (AB Biodisk, Solna, Sweden) in Mueller–Hinton (MH) agar. A spacer-free, two-stage exchange of the prosthesis with an 8 week interval was performed and antimicrobial chemotherapy was administered during the first 6 weeks.¹ The patient was treated with a 5.5 week course of vancomycin (1 g twice daily), followed by teicoplanin (400 mg once daily) for the remaining 4 days, after removal of the central venous catheter. On reimplantation, biopsies from periprosthetic tissue were obtained and vancomycin (1 g twice daily) was given for another 2 weeks, until cultures were reported to be negative.

Unfortunately, relapse occurred 4 months after reimplantation. Again, *S. epidermidis* was isolated, but showed two different phenotypes in susceptibility tests. While one strain was indistinguishable from the pathogen causing the primary infection, the second type was susceptible to oxacillin, but otherwise there was no difference in the resistance pattern. Both strains showed no increase in MIC of vancomycin, as revealed by Etest in MH agar. PCR for the *mecA* gene was negative in the second type, but positive in the original isolate. Interestingly, pulsed-field gel electrophoresis (PFGE) after *SmaI* digestion revealed only a slight difference between the two strains. The distance between two bands at ~140 kb and at ~160 kb was narrower in the *mecA*-positive strain (Figure 1), probably representing the location of the *mecA* gene. Vancomycin (1 g twice daily for 6 weeks) was administered during the implant-free-interval, and a complete recovery was made.

To our knowledge, *in vivo* loss of *mecA* gene in *S. epidermidis* after treatment has only been published in abstract form, without reporting any further details about antimicrobial agents and duration of therapy.³ Acquiring methicillin resistance is attributed to intra- and interspecies transfer of *SCCmec*,⁴ but little is known about the loss or deletion of the *mecA* gene. Long-term storage, high temperatures and UV radiation have been described to be factors influencing the stability of *SCCmec in vitro*.² Moreover, experiments have shown that coagulase-negative staphylococci exposed to glycopeptides may lose high-level resistance to oxacillin, without loss of the *mecA* gene.⁵ Recently published findings indicate that the acquisition and/or loss of *SCCmec* in *S. epidermidis*, may occur in the region of the *orfX* gene.⁶ Our case illustrates that the *mecA* complex may lose its stability after prolonged antimicrobial treatment.

Acknowledgements

We thank Reno Frei, MD and Professor Brigitte Berger-Bächi, PhD for their discussions regarding this case.

Transparency declarations

None of the authors has a conflict of interest regarding this case report. No funding was available.

References

1. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *New Engl J Med* 2004; **351**: 1645–54.
2. Adhikari RP, Scales GC, Kobayashi K *et al*. Vancomycin-induced deletion of the methicillin resistance gene *mecA* in *Staphylococcus aureus*. *J Antimicrob Chemother* 2004; **54**: 360–3.
3. Strandén A, Frei R, Widmer AF. *In vivo* deletion of the *mec* region in *S. epidermidis* in implant-associated infection. In: *Abstracts of the Forty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, 2002*. Abstract K-588, p. 313. American Society for Microbiology, Washington, DC, USA.
4. Wisplinghoff H, Rosato A, Enright MC *et al*. Related clones containing *SSCmec* type IV predominate among clinically significant *Staphylococcus epidermidis* isolates. *Antimicrob Agents Chemother* 2003; **47**: 3574–9.
5. Domaracki BE, Evans A, Preston KE *et al*. Increased oxacillin activity associated with glycopeptides in coagulase-negative staphylococci. *Eur J Clin Microbiol Infect Dis* 1998; **17**: 143–50.
6. Miragaia M, Couto I, de Lencastre H. Genetic diversity among methicillin-resistant *Staphylococcus epidermidis* (MRSE). *Microb Drug Resist* 2005; **11**: 83–93.

Journal of Antimicrobial Chemotherapy

doi:10.1093/jac/dki282

Advance Access publication 9 August 2005

The PROTEKT global study (year 4) demonstrates a continued lack of resistance development to telithromycin in *Streptococcus pneumoniae*

David J. Farrell* and David Felmingham

GR Micro Limited, 7–9 William Road, London NW1 3ER, UK

Keywords: *S. pneumoniae*, ketolides, macrolides

*Corresponding author. Tel: +44-20-73804469; Fax: +44-20-73887324; E-mail: d.farrell@grmicro.co.uk